Express Mail Label No.:
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Attorney Docket No. 34407

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS : See Attached Schedule A
ASSIGNEE : Trident Pharmaceuticals, Inc.
U.S. SERIAL NUMBER : See Attached Schedule A
U.S. PATENT NUMBER. See Attached Schedule A

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REVOCATION BY ASSIGNEE AND NEW POWER OF ATTORNEY

Trident Pharmaceuticals, Inc., owner of the United States patent applications and patents identified on the attached Schedule A, hereby revokes any and all former powers of attorney and hereby appoints the attorneys and/or agents associated with Mintz Levin Cohn Ferris Glovsky & Popeo, Customer Number 30623, as Applicants' attorneys with full power of substitution and revocation to take any and all action necessary with regard to the patent applications and patents identified on the attached Schedule A.

Please address all telephone calls to Sean M. Coughlin at telephone number (202) 585-3577. Please address all correspondence to **Customer No. 30623.**

Trident Pharmaceuticals, Inc., certifies under 37 C.F.R. § 3.73(b) that it is the Assignee of the right, title and interest in the patent applications and patents identified in the attached Schedule A by virtue of assignments of the patent applications and patents. Schedule A lists the reel and frame numbers for the assignment from the inventors to the University of Bristol. Attached, herewith, is an assignment of the applications and patents on Schedule A from the University of Bristol to Trident Pharmaceuticals, Inc.

I, the undersigned, am empowered to act on behalf of the Assignee. Acting on behalf of the Assignee, I have reviewed all the documents in the chain of title of the patent applications and patents on the attached Schedule A and, to the best of my knowledge and belief, title is in the Assignee identified above.

APPLICATION NO.: **Attached Schedule A**
ASSIGNEE: **Trident Pharmaceuticals, Inc.**

I, the undersigned, hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the patent.

Although Applicant believes no fees are due with this submission, the Commissioner is hereby authorized to charge any deficiency to Deposit Account No. 50-0311, Attorney Reference No. 34407 (Customer Number: 30623).

Respectfully submitted,

NAME: Robin Brown
TITLE: Director
COMPANY: Trident Pharmaceuticals, Inc.

Date:

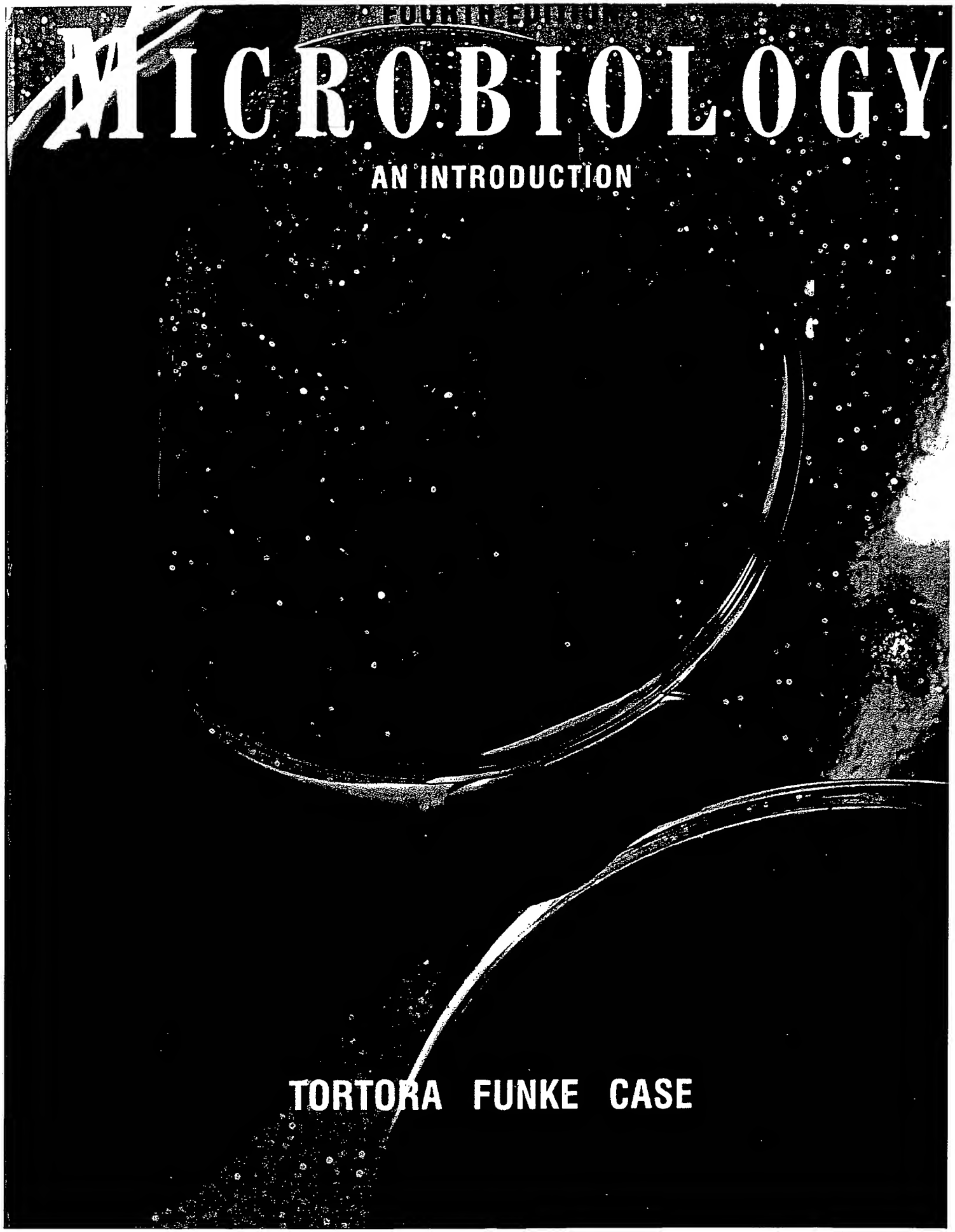
TRA 2198768v.1

Attorney Docket No.: 34407-501, -502, -503

SCHEDULE A

Title	Stage/Territory	Application and/or Patent No.	Reel/Frame No(s).
Therapeutic Agents and autoimmune diseases	PCT	PCT/GB96/01614	
	Australia	724516	
	Canada	2225788	
	China	96196258.5	
	Czech Republic	PV 12-98	
	Europe	EP 0841939	
	Hong Kong	HK 1006496	
	Hungary	224248	
	Japan	9-504927	
	Korea (South)	0452000	
	Mexico	9800241	
	Norway	319747	
	New Zealand	311762	
	Poland	187266	
	Russia	98101907	
	Singapore	50173	
	USA	08/999458; US 6,287,563	014066/0524; 014484/0401
	US-CIP	09/867,914	014070/0650
	US-CIP – Div 1	10/240,134	see 014070/0650
Agent for treating allergic or hypersensitivity condition	PCT	PCT/GB99/00070	
	Australia	762478	
	Canada	2,317,443	
	Europe	99900567.1	
	Japan	2000-527265	
	New Zealand	526218	
	USA	09/600,060	014070/0450
Vaccine	PCT	PCT/GB99/01461	
	Australia	2003261492	
	Canada	2331832	
	China	99808403.4	
	Czech Republic	PV 2000-4147	
	Eurasia	004794	
	Europe	99922284.7	
	Hungary	P 0104842	
	Israel	139467	
	India	IN/PCT/2000,00589/MUM	
	India	00353/MUMNP/2004 – a divisional application from	

Title	Stage/Territory	Application and/or Patent No.	Reel/Frame No(s).
		IN/PCT/2000,00589/MUM	
	Japan	2000-547996	
	Korea	2000-7012486	
	Mexico	010934	
	New Zealand	507911	
	Norway	20005599	
	Poland	P344519	
	Singapore	77072	
	South Africa	2000/6160	
	Ukraine	2000127088/M	
	USA	09/674,935	014273/0231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Composition and Film: York Graphic Services, Inc.
Printing and Binding: Rand McNally & Company
Cover photograph: © Will and Deni McIntyre/Allstock

Photograph acknowledgments appear following the Glossary.

Library of Congress Cataloging-in-Publication Data

Tortora, Gerard J.
Microbiology: an introduction/Gerard J. Tortora, Berdell R. Funke, Christine L. Case.—4th ed.
p. cm.
Includes bibliographical references and index.
ISBN 0-8053-8480-4
1. Microbiology. I. Funke, Berdell R. II. Case, Christine L., 1948– . III. Title.
QR41.2.T67 1991
576—dc20 91-26219
CIP

ISBN 0-8053-8480-4

3 4 5 6 7 8 9 10—RN—95 94 93 92

The Benjamin/Cummings Publishing Company, Inc.
390 Bridge Parkway
Redwood City, California 94065

TABLE 18.1 Principal Vaccines Used in Prevention of Bacterial Diseases in Humans

DISEASE	VACCINE	RECOMMENDATION	BOOSTER
Cholera	Crude fraction of *Vibrio cholerae*	For persons who work and live in endemic areas	Every 6 months as needed
Diphtheria	Purified diphtheria toxoid	See Table 18.3	Every 10 years for adults
Meningococcal meningitis	Purified polysaccharide from *Neisseria meningitidis*	For persons with substantial risk of infection	No booster effect with additional doses
Pertussis (whooping cough)	Killed *Bordetella pertussis*	Children prior to school age; see Table 18.3	For high-risk adults
Plague	Crude fraction of *Yersinia pestis*	For persons who come in regular contact with wild rodents in endemic areas	Every 6 to 12 months as needed
Pneumococcal pneumonia	Purified polysaccharide from *Streptococcus pneumoniae*	For adults with certain chronic diseases, persons over 65	No booster effect with additional doses
Tetanus	Purified tetanus toxoid	See Table 18.3	Every 10 years for adults
Tuberculosis	BCG vaccine, an attenuated strain of *Mycobacterium bovis*	For persons who are tuberculin-negative and who are exposed to tuberculosis for prolonged periods	Every 3 to 4 years as needed
Typhoid fever	Killed *Salmonella typhi*	For persons in endemic areas or areas having outbreak	Every 3 years as needed
Typhus fever	Killed *Rickettsia prowazekii*	For scientists and medical personnel in rural areas endemic for typhus	Every 6 to 12 months as needed
Hemophilus influenzae b meningitis	Capsular polysaccharide from *Hemophilus influenzae* b conjugated with protein to enhance effectiveness	See Table 18.3	At 12 or 15 months

physician, Jenner was intrigued by a dairymaid's assertion that she had no fear of smallpox because she had already had cowpox. Cowpox was a disease that caused lesions on cow udders; dairymaid's hands often became similarly infected during milking. Motivated by his childhood memory of variolation, Jenner began a series of experiments in 1798, in which he deliberately inoculated people with cowpox to prevent smallpox. This eventually led (in 1977) to the worldwide eradication of smallpox, the first disease for which this has been deliberately accomplished.

The development of conventional vaccines based on the model of the smallpox vaccine is the single most important application of immunology. Vaccines have greatly improved human health. Many pathogens transmitted by food or water can be controlled by sanitation or by antibiotics, if disease prevention fails. Viral diseases, however, are not readily treated once contracted, and transmission of viral pathogens by air or by direct contact is not easily prevented. Therefore, vaccination may be the only feasible method of controlling viral diseases. *Control* of a disease does not necessarily imply that everyone is immune to it. If most of the population is immune, outbreaks are limited to sporadic cases because there are not enough susceptible people to support the spread of epidemics. This is known as **herd immunity.**

CHARACTERISTICS OF VACCINES

A **vaccine** is a suspension of microorganisms (or some part or product of them) that will induce immunity in a host. These microorganisms may be either *inactivated* (killed) or only *attenuated*. In the latter case, they are still living but are so weakened or altered that they are no longer virulent; however, they will still provoke an immune response. **Toxoids** (inactivated bacterial toxins) will also induce immunity against their active forms.

Live, attenuated virus vaccines tend to mimic an actual infection and usually provide better immunity than that provided by inactivated viruses. Examples of live vaccines are the Sabin polio vaccines and those used against yellow fever, measles, rubella, and mumps. Many attenuated virus vaccines provide lifelong immunity without booster immunizations, and

TABLE 18.2 Principal Vaccines Used in Prevention of Viral Diseases in Humans

DISEASE	VACCINE	RECOMMENDATION	BOOSTER
Influenza	Inactivated virus	For chronically ill persons, especially with respiratory diseases, or for healthy persons over 65 years old	Annual
Measles	Attenuated virus	For infants 15 months old	Second dose before or during school years
Mumps	Attenuated virus	For infants 15 months old	
Rubella	Attenuated virus	For infants 12 to 19 months old, for females of childbearing age who are not pregnant	
Poliomyelitis	Attenuated or inactivated virus	For children, see Table 18.3; for adults, as risk to exposure warrants	
Rabies	Inactivated virus	For field biologists in contact with wildlife in endemic areas; for veterinarians	Every 2 years
Yellow fever	Attenuated virus	For persons traveling to endemic areas; for military personnel	Every 10 years
Hepatitis B	Subunit vaccine	Homosexual males, intravenous drug abusers, health workers exposed to blood	

*The duration of immunity is not known.

an effectiveness of 95% is not unusual. This long-term effectiveness probably occurs because the attenuated viruses tend to replicate in the body, and the original dose thereby increases considerably over time. One danger of such vaccines is that the live viruses can mutate to a virulent form, although this very rarely happens.

Viruses for vaccines may be inactivated by treatment with formalin or other chemicals. Heat is not used for this treatment because it is likely to alter the surface components of the virus and thus interfere with its ability to provoke an effective immune response. Commonly used inactivated virus vaccines include those used in humans against rabies (animals sometimes receive a live vaccine considered too hazardous for humans), influenza, and polio (the Salk polio vaccine).

In some vaccines, such as that for pneumococcal pneumonia, the antigens are the polysaccharide molecules of the bacterium's capsule. These vaccines must be readministered every few years, apparently because these antigens are less effective in stimulating antibody formation. Experience has also shown that vaccines against enteric bacterial pathogens, such as those causing cholera and typhoid, are not nearly as effective or long lived as those against viral diseases, such as measles and smallpox.

Vaccines that are effective against bacteria (including rickettsias and mycoplasmas) and against viruses have been produced, but to date no useful vaccines against chlamydias, fungi, protozoans, or helminthic parasites in humans are in use. However, researchers are working hard to develop a vaccine against malaria, which is caused by a protozoan parasite (see the box, p. 454).

The principal vaccines used to prevent bacterial and viral diseases in the United States are listed in Tables 18.1 and 18.2. Recommendations for the administration of some of them are given in Table 18.3. Travelers who might be exposed to cholera, yellow fever, or other diseases not endemic in this country will find that current inoculation recommendations are available from the U.S. Public Health Service and local public health agencies.

NEW VACCINE DEVELOPMENT

A basic problem with developing a new vaccine is the need for sufficient quantities of the organism. In some cases, this is very difficult—for example, when the pathogen does not grow in anything but a living human. The early successful vaccines used animal cultivation—for example, the vaccinia virus for smallpox was grown on the shaved bellies of calves, and the rabies virus was grown in the central nervous system of rabbits. The first vaccine against hepatitis B virus used viral antigens extracted from the blood of chronically infected humans because no other source was available. The successful development of cell culture methods for growing human viruses preceded the appearance in recent decades of the now familiar vaccines against polio, mumps, measles, and other

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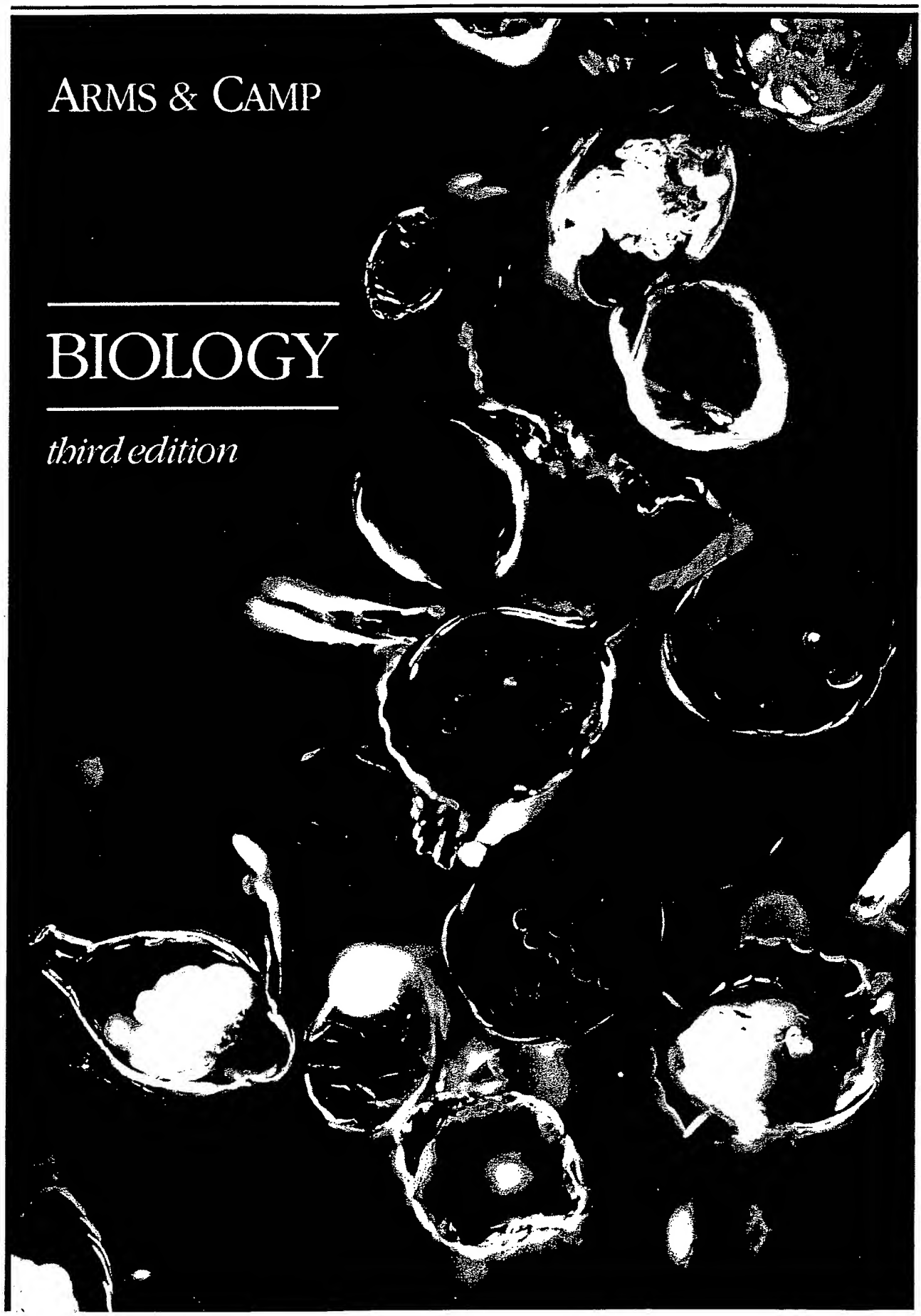

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Library of Congress Cataloging-in-Publication Data

Arms, Karen.
Biology.

Includes bibliographies and index.
1. Biology. I. Camp, Pamela S. II. Title.
QH308.2.A75 1986 574 86-10046
ISBN 0-03-003644-5

BIOLOGY 0-03-003644-5

Library of Congress catalog card number 86-10046.

789 071 9876543

CBS COLLEGE PUBLISHING
Saunders College Publishing
Holt, Rinehart and Winston
The Dryden Press

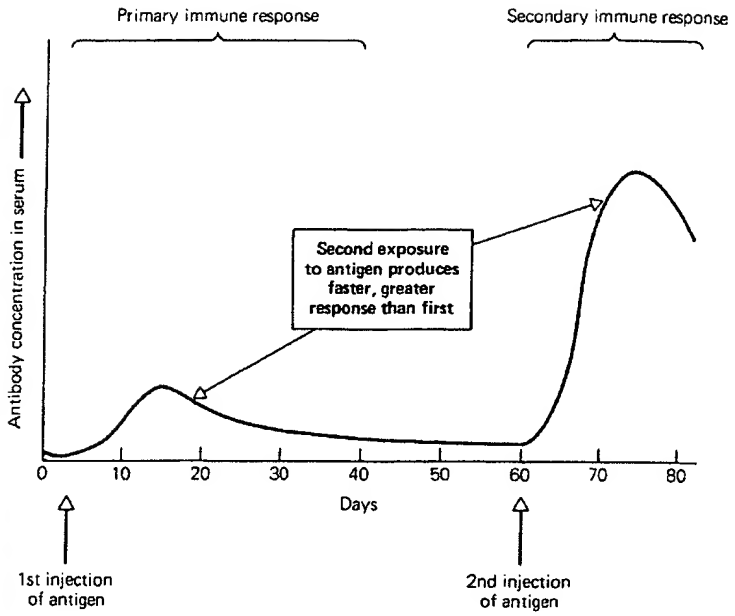

FIGURE 35–7 Primary and secondary immune responses. The graph shows the amount of antibody to a specific antigen detected in the blood of a rabbit. Colored arrows below the graph indicate the times of the first and second injections of antigen. The second time the antigen is injected, the rabbit produces the specific antibody more rapidly and in greater amounts.

35-F PRIMARY AND SECONDARY IMMUNE RESPONSES

The immune response to the body's first encounter with a foreign antigen is called a **primary immune response** to that antigen (Figure 35–7). Most such responses involve both cellular and humoral mechanisms. During a primary response, the antigen will eventually disappear from the blood, bound by antibody and eaten by macrophages. Then "suppressor" T lymphocytes cause the clone of B lymphocyte cells that is producing antibodies to stop dividing. The clone does not die out, however; it remains in the body, an enlarged population of B cells that react to that particular antigen. As a result, if the same antigen enters the body again, these cells mount a **secondary immune response,** faster and more extensive than the primary response, which quickly eradicates the threat (Figure 35–7).

Once the immune system has made a primary response to an antigen, it retains a "memory" of that antigen. In the humoral immune response, immunological memory consists of the enlarged clone of B lymphocytes sensitized to the antigen. In the case of cell-mediated responses, we know less about the memory.

Because each B lymphocyte produces only one, or at most a few, types of antibody, the body must build up a memory clone for each antigen it encounters before it has an arsenal of secondary responses to most of the microorganisms it encounters. This is why babies have so many colds and infections in their first few years: they must encounter many antigens, and build up many clones of memory cells, before they are immune to as many diseases as the average adult.

35-G VACCINATION

Vaccination against a specific disease produces a primary immune response and thereby creates an immunological memory, ready to trigger an efficient secondary response at the body's first real battle against the disease antigen. The practice of vaccination, however, began long before people understood how it works. Arabic and Chinese manuscripts more than a thousand years old refer to vaccination against smallpox. The wife of the British ambassador to Turkey introduced this ancient custom into England in 1718. She vaccinated her daughter by rubbing part of the scab from a healed smallpox sore into a small wound in the skin. This introduced a few live smallpox viruses into the body, stimulating a primary immune response and thereby conferring immunity to smallpox in later life. The snag, of course, is that vaccination with even a small amount of live virus might cause a full-blown, and possibly fatal, case of smallpox. Edward Jenner, an English physician, found a

way around this problem in 1796. Jenner noticed that dairy workers who had caught the relatively mild disease cowpox from cows seemed to be immune to smallpox. He found that rubbing pus from cowpox sores into scratches in the skin prevented people from coming down with smallpox later. In this case, the antigens of smallpox and cowpox are so similar that the same antibodies work against them both. Almost a century later, Louis Pasteur (who introduced the word "vaccine") found a safer way to prepare vaccines. He discovered how to **attenuate** microorganisms, damaging them by heat and other treatments, until they could no longer cause disease.

Nowadays, we have vaccines for a number of bacterial and viral diseases, including polio and influenza, which proved especially difficult. "Booster shots" serve to jog the body's immunological memory into producing more antibodies and more cells, ensuring that there are plenty of memory cells available if a diphtheria or whooping cough bacterium should invade.

Several important diseases remain without effective vaccines, including trypanosomiasis and malaria (Chapter 24). Trypanosomes produce many different antigens, always keeping one jump ahead of the immune system (and vaccine manufacturers). Malaria organisms (*Plasmodium* species) change their surface antigens at different stages of the life history (see Figure 24–8). They also shed their surface layers as fast as host antibodies bind, leaving only their empty coats to be engulfed by phagocytes.

Smallpox was not only the first disease to be prevented by vaccination but also the first disease to be officially declared eradicated by human efforts. The last known outbreaks of smallpox occurred in the Indian subcontinent and Africa in the late 1970s. Large-scale international vaccination programs greatly reduced the annual number of smallpox cases, but the disease persisted for many years at low levels. The final conquest came after health officials adopted a different strategy: searching out pockets of infection (people were given reward money for each case they reported), quarantining the victims, and inoculating their friends and relations.

35-H PASSIVELY ACQUIRED IMMUNITY

An animal is said to be passively immune when it contains antibodies that were not synthesized in its own body. A newborn baby is passively immune, temporarily protected from disease by immunoglobulins that reach it from the mother's blood before birth. These maternal antibodies are steadily used up over a period of a few months until the baby's immune system is sufficiently mature to take over.

The breast-fed newborn is also protected by colostrum, a thin fluid produced by the mammary glands after childbirth before the flow of milk begins. Colostrum contains antibodies believed to protect the human infant's digestive tract from infections. Once the normal bacterial inhabitants of the digestive tract become established, they themselves suppress the invasion of dangerous newcomers. Human babies do not absorb antibodies from colostrum into the blood, although the young of some other mammals do.

Passive immunity can also be used medically. Some antigens are so virulent that the body's own primary immune response has little chance of averting serious damage or death. If by some mischance such an antigen enters the body, the victim can sometimes be protected temporarily by injections of antibodies produced by another animal. These antibodies are usually prepared by giving several small injections of an antigen to a horse and later collecting samples of the horse's blood, which now contains antibodies to that antigen. The horse's serum can then be stored until it is needed to protect a patient from that specific antigen, such as tetanus or snake venom. Such injections should not be used lightly, however, because the recipient will produce an immune response to the horse proteins in the serum; this might produce a dangerous secondary reaction if the patient were ever again injected with horse serum.